British Journal of Industrial Medicine 1986;43:243–256

Investigation of the potential carcinogenicity of a range of chromium containing materials on rat lung

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ABSTRACT Twenty one chromium containing materials were examined for carcinogenic activity in a two year study using an intrabronchial pellet implantation system whereby pellets loaded with test material were surgically implanted into the lower left bronchus of rats. The principal aim of the study was to extend our knowledge of the carcinogenic potential of chromium compounds and, in particular, chromates (Cr^{6+}). A statistically significant incidence of treatment related lung tumours was found with some sparingly soluble chromate materials. All tumours were large keratinising squamous carcinomas of the left lung, except for a single left lung adenocarcinoma and two left lung anaplastic carcinomas. No bronchial carcinomas (0/100) were seen in the negative control group (blank pellet loaded with cholesterol), whereas bronchial carcinomas (22/48 and 25/100) occurred in the two positive control groups which received pellets loaded with 20-methylcholanthrene and calcium chromate respectively. Among the 20 test materials, only three groups gave statistically significant numbers of bronchial carcinomas. Two of these were groups receiving different samples of strontium chromate which gave 43/99 and 62/99 tumours. The third group, zinc chromate (low solubility), gave 5/100 bronchial carcinomas. A further zinc chromate group (Norge composition) produced 3/100 bronchial carcinomas which was not statistically significant. A few lung tumours were observed in other test groups.

The toxicological effects of exposure to chromium containing materials have been known since the early nineteenth century. Their irritant and corrosive action on the skin and nasal mucosa have been widely reported, and in the United Kingdom notification of cases of chrome ulceration to HM Chief Inspector of Factories has been a statutory obligation since 1919. The first reported case of cancer associated with chromates was in 1890[1] and was that of an adenocarcinoma of the nasal turbinates in a chrome pigment worker from Scotland.

Early observations of lung cancer in German workers in the bichromate producing industry were made in 1911 and 1912 but not published until 1932.[2] Later observations were made in the German industry throughout the 1930s[3] but only since the late 1940s have epidemiological surveys conducted in the United States of America, the United Kingdom, and elsewhere attempted to quantify the risk.[4-8]

These studies showed that an increased risk of lung cancer existed for workers engaged in manufacturing bichromates (Cr^{6+}) from the ore, chromite (Cr^{3+}). The industrial production of bichromates potentially exposes the process workers to chrome ore, calcium chromate, sodium and potassium chromate, and dichromate and chromic acid as well as other intermediate materials. In the mid 1970s the risk of bronchial carcinoma was shown to occur also among chrome pigment makers.[9-11] The chrome pigments include materials used to impart colour, mainly greens, yellows, and oranges, from a variety of lead chromate containing pigments, with additives to produce the various shades required, as well as zinc chromate containing pigments. A second major use of a particular group of chromate pigments is in coatings designed to prevent the corrosion of metals. This group includes zinc and strontium chromates. In other groups of workers exposed to chromium containing materials—for instance, chrome platers,[12-15] chromate spray painters,[16,17] and stainless-steel welders[18]—reports are sparse and inconclusive. The most recent evaluation of the carcinogenic risk of chromium and its compounds by the International Agency for Research on Cancer (IARC) in 1982[19] is:

Accepted 5 August 1985

"A *Evidence for carcinogenicity to humans (sufficient)*
An increased incidence of lung cancer has been observed among workers in the chromate producing industry and possibly also among chromium platers and chromium alloy workers. There is a suggestion that cancers at other sites are also increased in such populations. The chromium compound(s) responsible has not been specified.

B *Evidence for carcinogenicity to animals (sufficient)*
Calcium chromate is carcinogenic to rats after its administration by several routes, including intrabronchial implantation. Chromium chromate, strontium chromate, and zinc chromate produce local sarcomas in rats at the sites of their application. Inadequate evidence was available for the carcinogenicity in mice and rats of barium chromate, lead chromate, chromic acetate, sodium dichromate, and chromium carbonyl."

These conclusions are based on IARC's interpretation of the experimental and epidemiological evidence then available. Later studies[20 21] and data presented in the present paper indicate that a more precise evaluation of carcinogenic potential may now be possible.

To identify the Cr^{6+} compound(s) that may have caused the increased risk of lung cancer in the manufacture of bichromates and chrome pigments, animal models have been used with a wide variety of routes of application but few directly to the lung itself. A more specific animal experiment was a study in which a series of chromium containing materials encountered in the bichromate producing industry were tested, using the rodent lung intrabronchial pellet implantation technique.[22] Both calcium chromate and zinc potassium chromate (a chrome pigment not occurring in the industry but included to test a solubility theory) induced lung tumours (8/100 and 3/100 respectively). Both results were statistically significant ($p < 0.05$).

The present study was undertaken to complement these earlier findings using the same experimental technique but applied to 20 chromium containing compounds including seven from the bichromate producing industry and 13 made and used in the chrome pigment industry. The nature of the materials tested is such that the results may well be relevant to other chromium using industries including chromium plating and spray painting.

Materials and methods

The technique used in the present study was modified from that originally developed and described by Kuschner *et al* and referred to as the "intrabronchial pellet implantation procedure" in which a metal wire basket or pellet containing the test material is surgically implanted into the left bronchus of an anaesthetised rat.[23] The metal mesh acts as a framework in and around which the test material, mixed with cholesterol, is suspended and from which it leaches. This enables a selected zone of bronchial epithelium to be exposed to a putative carcinogen for a continuing period. The animals were studied for two years, after which time the survivors were killed and the lungs and other organs presenting abnormalities at necropsy were examined microscopically. The procedure for coating the pellets was modified from that used previously since the stability of the chrome pigments might be altered when heated for a period in excess of two minutes. Test material (10 g) was added to cholesterol (10 g) and placed in a glass bottle. After thorough mixing, aliquots (0·5 g) were taken and heated to 160°C to give a molten mixture into which the preweighed pellet was dipped. Each pellet was held by the hooks, suspended into the molten material for ten seconds, taken out, and allowed to solidify. In this way five pellets were coated in the two minute heating period allowed for each aliquot. Excess material was removed from the body of the pellet by fitting it into an implantation trochar. The coated pellet was then reweighed and the exact weight of test material in and around each pellet calculated. All pellets were kept in individual bijou bottles and sterilised before surgical implantation into the left bronchus. Equal numbers of male and female random bred eight to ten week old Porton-Wistar rats (100 per group) obtained from A Tuck & Sons Ltd, Rayleigh, Essex, were used in all experiments. Autoclaved cubed diet (rat and mouse breeding diet, Pilsburys) and drinking water were available ad libitum.

Table 1 lists the test materials which were provided by the bichromate producing and chrome pigment industries. The study was conducted in accordance with the guidelines published by the Food and Drug Administration (FDA) in a document entitled "Nonclinical laboratory studies—proposed regulations for good laboratory practice" (1976).

The pathological evaluation of the bronchial changes evoked by this technique were broadly classified as inflammatory, preneoplastic, and neoplastic. Independent re-evaluation of suspect neoplastic lung lesions and a selection of normal lungs was performed by an external pathologist.

In the case of lung tumour data the statistical analysis was similar to a previous study and was based on the "null hypothesis" that none of the test substances is carcinogenic.[22] Each animal will therefore have had the same probability of developing a bronchial carcinoma regardless of the chemical on the pellet. The hypothesis includes the negative

Table 1 *Characterisation of chromium containing materials*

	Group No	*Commercial description*	*Cr^{3+} Cr^{6+}*	*Physical appearance*	*Water soluble (cold) Cr^{6+} content* (ppm Cr)*	*Composition of material Chromium & other major components*	*%*
P	1	Lead chromate (pure)	6	Yellow/orange powder	1.7×10^1	Pb CrO_4	64·0 35·8
P	2	Primrose chrome yellow	6	Yellow powder	5·0	Pb Cr	62·1 12·6
P	3	Strontium chromate	6	Yellow powder	2.07×10^5	Sr CrO_4	42·2 54·1
P	4	Barium chromate (98%) tech	6	Lemon powder	1.1×10^1	Ba CrO_4	54·1 42·1
P	5	Molybdate chrome orange	6	Orange/red powder	Less than 1·0	Pb Cr	62·9 12·9
P	6	Zinc chromate 1W (low water solubility)	6	Yellow powder	4.2×10^2	ZnO CrO_3	39·4 40·8
P	7	Zinc tetroxychromate	6	Yellow powder	2.3×10^2	Zn Cr	56·6 8·8
P	9	Light chrome yellow	6	Yellow powder	1·0	Pb Cr	62·1 12·5
P	10	LD chrome yellow supra 70FS	6	Yellow/orange powder	Less than 1·0	PbO CrO_3	61·5 26·9
C	11	TSS 711 Calcium chromate Positive control	6	Yellow powder	1.81×10^5	$CaCrO_4$ Na_2CrO_4	96·7 0·8
B	12	TSS 613 chromic acid flake	6	Red/black flakes	4.0×10^5	CrO_3	99·9
P	13	Medium chrome yellow	6	Yellow powder	2·0	Pb Cr	60·2 16·3
P	14	Zinc chromate LD-KSH/SM (Norge)	6	Yellow powder	6.4×10^4	ZnO CrO_3	39·2 43·5
B	15	TSS 612 sodium dichromate dihydrate	6	Orange crystals	3.28×10^5	$Na_2Cr_2O_7$ $2H_2O$	99·7
B	16	TSS 643D High lime residue from old tip	6 + 3	Light brown powder	1.82×10^3	Cr_2O_3 $CaCrO_4$ Na_2CrO_4	2·4 2·7 1·0
B	17	TSS 643A Vanadiam solids from leaching plant	6	Lemon/green powder	5.4×10^4	$CaCrO_4$ Na_2CrO_4	5·3 17·2
B	18	TSS 695 High silica chrome ore	3	Brown/grey powder	5·0	Cr_2O_3	46·1
B	19	TSS 643B Kiln frit—2% lime in feedmix'	6 + 3	Dark brown powder	8.46×10^4	Cr_2O_3† Na_2CrO_4	13·0 29·0
B	20	TSS 643C Recycled residue—2% lime in feed mix	6 + 3	Dark brown powder	6.0×10^3	Cr_2O_3 Na_2CrO_4	20·4 2·2
P	21	Medium chrome yellow—silica encapsulated	6	Yellow powder	1.7×10^1	Pb Cr	40·4 10·5
P	22	Strontium chromate	6	Yellow powder	6.3×10^4	Sr Cr	43·0 24·3
C	8	Cholesterol (pure) Negative control		White powder	Koch Light Labs, Colnbrook, England	5(6)-Cholesten-3β-ol	
C	23	3-Methyl cholanthrene Positive control		Yellow crystals	Sigma Chem Co, Poole, England	20-Methylcholanthrene	

*Method used was: British Standards Institution BS3483: Part C2: 1980 for testing pigments for paints. Determination of matter soluble in water (cold extraction method); in conjunction with International Standard ISO 3856/5-1980(E). Appendix D. Part 5: Determination of hexavalent chromium content of the pigment and extender portion of the paint—diphenylcarbazide spectrophotometric method.
P = Materials encountered in the chrome pigment industry.
B = Materials encountered in the bichromates producing industry.
C = Control materials.
†This material contained 0·7% CaO which is equivalent to 1·95% $CaCrO_4$. In practice most of the calcium would be present as the chromate.

control group 8 but not the positive control groups (group 11, calcium chromate and group 23, 20-methylcholanthrene). The rationale behind the null hypothesis is based on the assumption that the occurrence of a primary bronchial carcinoma is rare. The evaluation of the bronchial carcinoma data assumes a Poisson distribution for the occurrence of lung cancer in rats, and our experience of zero tumours in over 500 control animals receiving blank pellets loaded with cholesterol supports this assumption. Thus an "expected" incidence of lung tumour per group (E) may be calculated from the number of observed bronchial carcinomas (O) that are found in the total number (test groups plus negative control group) of rats. The calculation of probability is similar to that used in a previous study[22] and is in accordance with the general principals set out in the IARC monograph on screening assays for carcinogens.[24]

Results

GENERAL OBSERVATIONS

The animals remained in good general health during the two year period of the experiment with 95·7%

alive at 400 days and an overall survival at 700 days of 53·9%.

Student's *t* test was performed on the weight data for each treatment group and compared with the negative control group (group 8); there was no significant increase or decrease in body weight in test groups.

Lung tumours first appeared in the positive control groups (group 23–20 methylcholanthrene) on day 294 (male rat) and group 11 (calcium chromate) on day 439 (male rat). In groups 3 and 22 (both strontium chromate) the first lung tumour appeared in group 3

Table 2 *Summary of all lung findings after macroscopic and microscopic examination*

Group No	*Test material*	*No rats in group*	*No lungs examined*	*No lungs with chronic inflam**	*(M)*	*(F)*	*No lungs with bronchial inflam†*	*Squamous metaplasia‡*	*(M)*	*(F)*
1	Lead chromate (pure)	100	98	4	(2)	(2)	93	16	(7)	(9)
2	Primrose chrome yellow	100	100	5	(5)		89	14	(9)	(5)
3	Strontium chromate	100	99	10	(8)	(2)	36	13	(3)	(10)
4	Barium chromate	101	101	13	(9)	(4)	87	12	(6)	(6)
5	Molybdate chrome orange	100	100	10	(4)	(6)	90	13	(6)	(7)
6	Zinc chromate (low sol)	100	100	8	(4)	(4)	76	13	(7)	(6)
7	Zinc tetroxychromate	100	100	15	(12)	(3)	82	6	(3)	(3)
8	Cholesterol	100	100	7	(4)	(3)	89	7	(3)	(4)
9	Light chrome yellow	100	100	2	(1)	(1)	94	15	(10)	(5)
10	LD chrome yellow	100	100	2	(1)	(1)	92	11	(6)	(5)
11	Calcium chromate	100	100	5	(3)	(2)	60	9	(4)	(5)
12	Chromic acid	100	100	7	(5)	(2)	79	9	(5)	(4)
13	Medium chrome yellow	100	100	5	(5)		80	13	(7)	(6)
14	Zinc chromate (Norge)	100	100	4	(4)		86	4	(2)	(2)
15	Sodium dichromate	100	99	20	(8)	(12)	72	14	(6)	(8)
16	High lime residue	100	99	16	(11)	(5)	74	10	(4)	(6)
17	Vanadium solids	100	100	44	(22)	(22)	50	10	(5)	(5)
18	High silica chrome ore	101	99	7	(6)	(1)	92	7	(3)	(4)
19	Kiln frit (+2% limestone)	100	100	13	(7)	(6)	79	6	(3)	(3)
20	Recycled residue (+2% limestone)	100	100	12	(8)	(4)	82	13	(3)	(10)
21	Silica encaps medium chrome yellow	100	100	9	(4)	(5)	92	17	(7)	(10)
22	Strontium chromate	100	99	3		(3)	25	7	(1)	(6)
23	20-methylcholanthrene	48	48	2	(1)	(1)	19	8	(3)	(5)

*Chronic inflammation includes all changes of an inflammatory nature, either of micro/macroscopic description. Lung tumours with such changes are excluded.

†Bronchial inflammation is scored where this is the major inflammatory response. It varied from very slight to severe epithelial changes (microscopic).

‡This condition was scored for all lungs exhibiting squamous metaplasia except those with squamous carcinoma.

on day 340 (female rat) and in group 22 on day 291 (male rat). Thereafter lung tumours continued to appear until the end of the study, with 18/172 appearing only at the terminal kill (2 years), whereas the remainder (154/172) were considered to be the direct cause of death of the animals in which they occurred.

PATHOLOGY OF LUNG LESIONS

The relevant findings from the macroscopic and microscopic examinations of the lungs are summarised in table 2. As expected, there were various tissue responses to the implanted pellets and test materials but these fell well into the pathological catego-

Dysplasia (M) (F)	*Carcinoma in situ* (M) (F)	*Primary bronchial carcinoma* (M) (F)	*Other primary lung tumours* (M) (F)	*Malignant lymphoma* (M) (F)	*Secondary tumour deposits in lung* (M) (F)
0	0	1 × scc; M (1)	0	1; F (1)	0
2; F (2)	0	1 × scc; M (1)	1 × fis; M (1)	0	0
1; M (1)	0	43 × scc; M (20), F (23)	0	0	0
2; F (2)	0	0	0	0	0
4; M (1), F (3)	0	0	0	0	0
2; M (2)	0	5 × scc; M (3), F (2)	0	0	0
1; M (1)	0	1 × scc; M (1)	0	0	0
0	0	0	0	0	1 × Phaeochromocytoma; M (1)
2; M (2)	0	0	0	0	1 × Uterine ac; F (1)
0	0	1 × scc; M (1)	0	0	0
1; F (1)	0	24 × scc; M (12), F (12) 1 × ac; F (1)	0	2; M (2)	1 × Intra-abdominal ac; F (1)
2; M (1), F (1)	0	1 × scc 1 × anc; F (2)	0	0	0
0	0	1 × scc; M (1)	0	0	0
0	0	3 × scc; M (2), F (1)	0	0	1 × Directly invading thymoma; F (1)
2; M (2)	0	1 × scc; M (1)	0	0	0
0	0	1 × scc; M (1)	0	0	1 × Bilat anc from abdominal area; M (1)
1; M (1)	0	1 × scc; M (1)	0	0	0
3; M (1), F (2)	0	0	0	0	1 × Uterine ac; F (1)
2; F (2)	0	2 × scc; F (2)	0	1; M (1)	1 × Uterine ac 1 × Intra-abd ac 1 × Mam ac; F (3)
1; F (1)	0	0	0	0	0
3; M (1), F (2)	0	0	0	0	0
1; F (1)	0	62 × scc; M (30), F (32)	0	0	0
3; M (1), F (2)	1; F (1)	21 × scc; M (14), F (7) 1 × anc; F (1)	0	0	0

fis = Fibrosarcoma.
scc = Squamous carcinoma.
ac = Adenocarcinoma.
anc = Anaplastic carcinoma.
mam ac = Mammary adenocarcinoma.
uter ac = Uterine adenocarcinoma.
intra abd ac = Intra-abdominal adenocarcinoma.
bilat anc = Bilateral anaplastic carcinoma from abdominal area.

ries described in the table. Particular care was taken to distinguish between inflammatory, preneoplastic, and neoplastic response. The left lung bronchial carcinomas recorded in this table tended to affect a major part of the left lung. Inflammatory and metaplastic changes of the lung and bronchus (figs 1–3) did not appear to show any treatment relationship apart from a high incidence of chronic parenchymal inflammatory changes in group 17 (vanadium solids), presumably related to the presence of calcium vanadate ($Ca_3(VO_4)_2$) which may hydrolyse to produce vanadium pentoxide, a well known lung irritant.

PRIMARY BRONCHIAL CARCINOMAS

These lesions were invariably keratinising squamous carcinomas (figs 4 and 5) of the left lung with varying degrees of necrosis. Occasionally metastases to the kidney or hilar lymph nodes were seen, as was invasion of the right lung or diaphragm. One adenocarcinoma was observed (fig 6) in group 11, calcium chromate, and two lesions were diagnosed as anaplastic carcinomas (fig 7), one in group 12, chromic acid and one in group 23, 20-methylcholanthrene.

In this study the "induction period" was used to denote the time in days from the surgical insertion of the pellet to the observation of a lung tumour at postmortem examination. Table 3 gives a summary of the bronchial carcinoma data. This shows clearly the differing carcinogenic potential of the various chromium containing (Cr^{6+}) materials tested by this system.

Discussion

LUNG TUMOURS AND EXPERIMENTAL TREATMENT

Our experience of the technique, and that of Laskin *et al*[25] shows that three basic criteria must be met to establish a causal link between the development of lung tumours and treatment. These are: (1) that the neoplasm should be a bronchial carcinoma (squamous carcinoma, adenocarcinoma, or anaplastic carcinoma); (2) the tumour should originate in the left lung; and (3) that it should occur in the latter part (after 250 days) of the animal's life. All lung tumours in table 3 satisfied these criteria and are thus considered to be related to treatment.

INTERPRETATION OF LUNG TUMOUR DATA

In a previous study (table 4) it was shown that in a series of 14 chromium containing materials only three

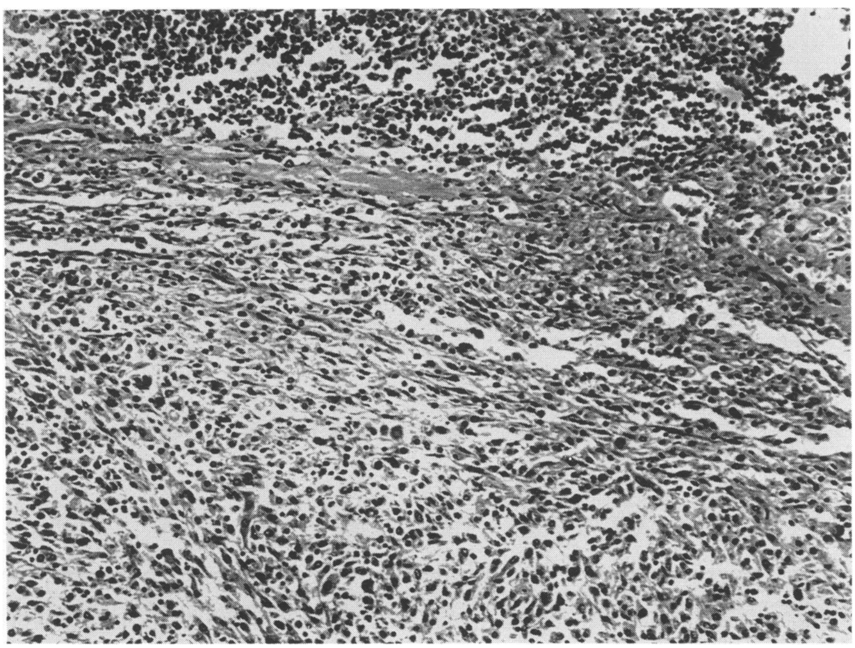


Fig 1 *Section from edge of an abscess with necrotic debris surrounded by inflamed granulation tissue. Some fibroblasts are pleomorphic. Fibroblastic responses in such lesions are often intense and zones of well formed metaplastic bone are not uncommon. Another feature is regenerative proliferation of bronchiolar epithelium which may simulate invasive carcinoma. (H & E ×130.)*

Fig 2 *Example of mild bronchial inflammation. Mucosa is thickened with polypoid folds and lining ciliated columnar epithelium is slightly hyperplastic. Mixed inflammatory cells are present in submucosa, extending to about depth of submucous glands. (H & E × 130.)*

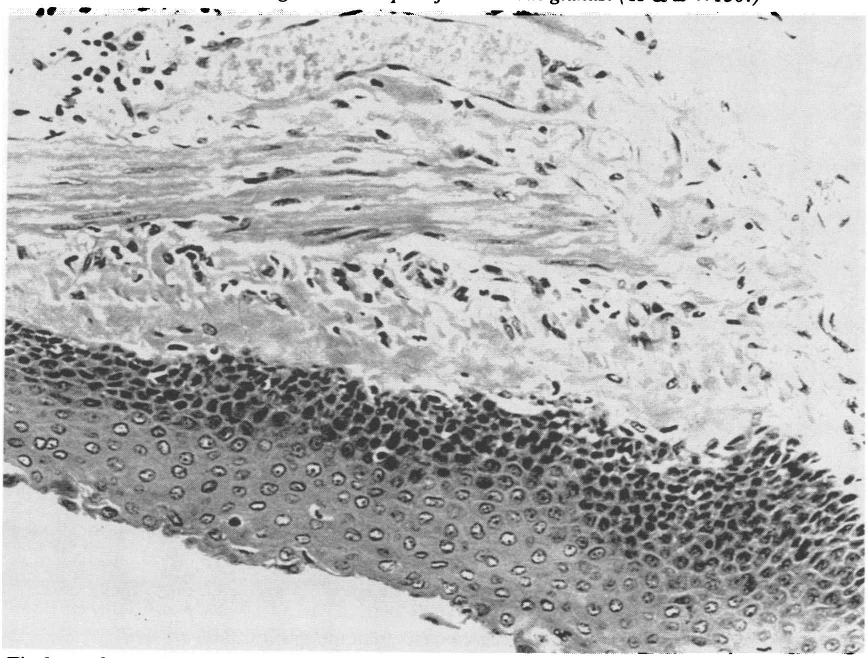
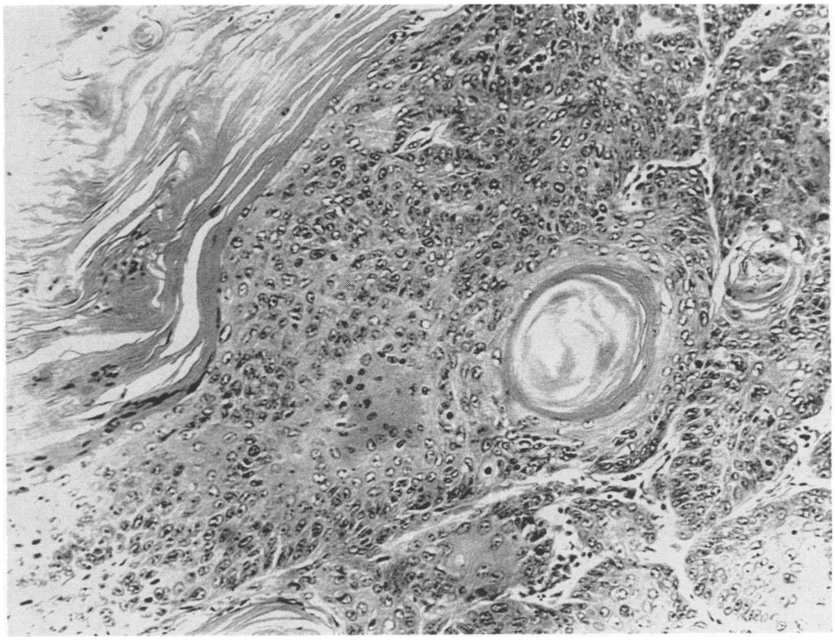
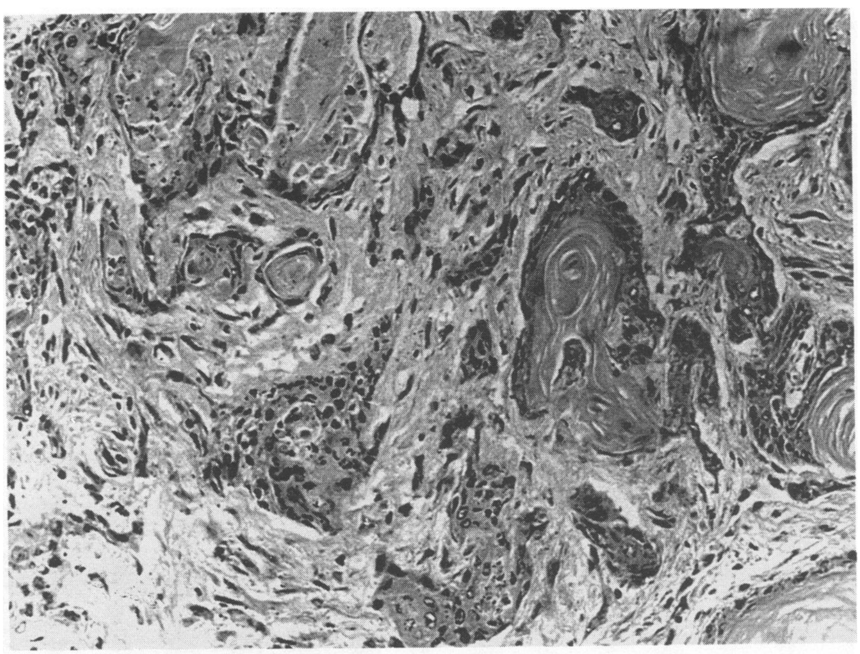


Fig 3 *A focus of squamous metaplasia which has completely replaced normal ciliated columnar epithelium. Cells are stratified and appear to be maturing from basal layer outwards as in normal squamous epithelium—for example, in oesophagus or cervix uteri. No keratin layer is seen here but some foci of squamous metaplasia are heavily keratinised. Mitotic figures, seen at higher power, are few and morphologically normal. (H & E × 210.)*





Figs 4 and 5 *Two bronchial squamous carcinomas from test groups. Both are well differentiated, keratinising lesions. Tumour shown in fig 5 has a rather more dispersed, infiltrated growth pattern. No distinctive morphological features were identified in squamous carcinomas from any of the test groups or from two positive control groups. Both tumours illustrated here are histologically identical to squamous carcinomas of bronchus in man. (Both H & E × 130 and × 210.)*

Fig 6 *Primary adenocarcinoma of bronchus, showing clear acinar growth pattern. This is a rare tumour in rats. Histologically it is similar to adenocarcinoma of lung in man where such tumours have certain distinctive clinical features: a greater incidence among women than men, a tendency to develop in peripheral parts of bronchial tree, and a weak association with inhaled carcinogens (except perhaps asbestos). (H & E × 130.)*

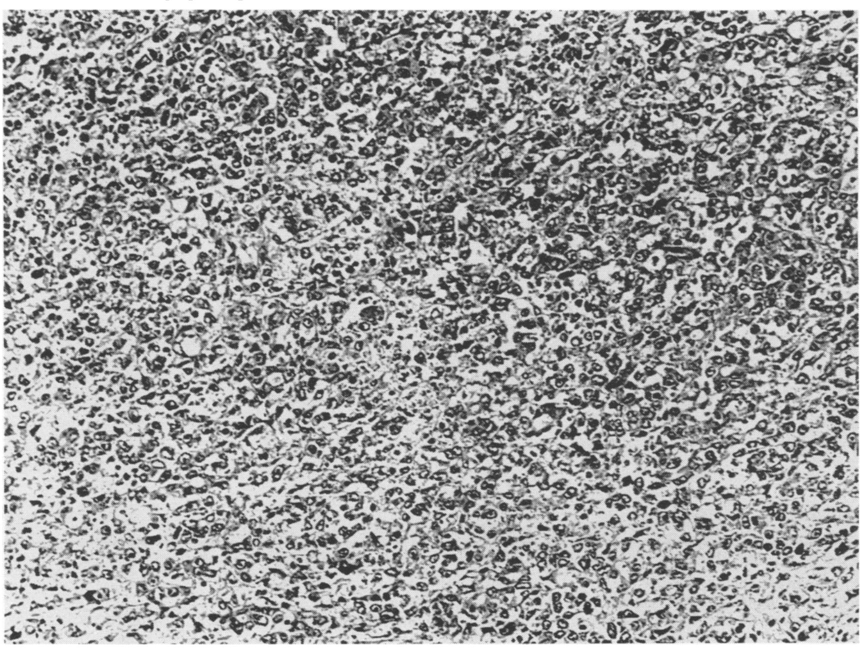


Fig 7 *A primary anaplastic carcinoma of bronchus. It may be classified as a small cell lesion (cf WHO scheme) but histological appearances do not resemble an oat cell carcinoma, the commonest form of small cell anaplastic carcinoma in man. Anaplastic tumours are rare in rats and, of the three main tumour types, they have least resemblance to their human counterparts. (H & E × 130.)*

produced bronchial carcinomas. These three materials were hexavalent chromates. Only two gave statistically significant numbers of tumours, however, and these (calcium chromate and zinc potassium chromate) may be described as sparingly soluble. A single tumour was also produced with chromic acid.

In the present study, which has included a wider range of hexavalent chromium containing materials, it is convenient to discuss the results as follows (for an overall comparison see table 3).

Strontium chromates

The high incidence of bronchial carcinomas (43/100-group 3 and 62/100-group 22) seen in rats treated with strontium chromate is so obvious that statistical tests for significance are not needed to conclude that this material is a strong carcinogen under these study conditions. The reason for the difference in tumour incidence between the two strontium chromate groups may be due to differences in the composition of the pigment (crystal structure and particle size, for example, since the materials came from separate suppliers) or it may be a function of the inherent variability of the experimental method. The only other reported studies with strontium chromate are those of Hueper in which he produced 17/28 intrathoracic tumours (type unstated) after intrapleural injection and 15/33 local intramuscular sarcomas after implantation.[26]

Table 3 *Summary of bronchial carcinoma data*

Group No	*Test material*	*No lungs examined*	*No bronchial carcinomas*	*(M)*	*(F)*	*Probability**	*Significance*	*Mean induction period (+/− SD) in days*	
1	Lead chromate (pure)	98	1	(1)		0·37	NS	502	
2	Primrose chrome yellow	100	1	(1)		0·37	NS	673	
3	Strontium chromate	99	43	(20)	(23)	—	—	567·3	(119·4)
4	Barium chromate	101	0			—	—	—	
5	Molybdate chrome orange	100	0			—	—	—	
6	Zinc chromate (low sol)	100	5	(3)	(2)	0·004	S	599·8	(115)
7	Zinc tetroxychromate	100	1	(1)		0·37	NS	623	
8	Cholesterol	100	0			—	—	—	
9	Light chrome yellow	100	0			—	—	—	
10	LD chrome yellow	100	1	(1)		0·37	NS	417	
11	Calcium chromate	100	25	(12)	(13)	—	—	576·4	(98·8)
12	Chromic acid	100	2		(2)	0·19	NS	580	(77·8)
13	Medium chrome yellow	100	1	(1)		0·37	NS	591	
14	Zinc chromate (Norge)	100	3	(2)	(1)	0·068	NS	606	(173)
15	Sodium dichromate	99	1	(1)		0·37	NS	658	
16	High lime residue	99	1	(1)		0·37	NS	726	
17	Vanadium solids	100	1	(1)		0·37	NS	645	
18	High silica chrome ore	99	0			—	—	—	
19	Kiln frit (2% limestone)	100	2		(2)	0·19	NS	573·5	(47·5)
20	Recycled residue (2% limestone)	100	0			—	—	—	
21	Silica encaps medium chrome yellow	100	0			—	—	—	
22	Strontium chromate	99	62	(30)	(32)	—	—	542·5	(116·3)
23	20-Methylcholanthrene	48	22	(14)	(8)	—	—	575·2	(134)

Number of animals per group was 100 except in groups 4 and 18 which had 101 and group 23 which had 48.

S = Statistically significant at the 5% level.
NS = Not statistically significant at the 5% level.
* = The probability of observed numbers of tumours in each test group may be calculated from the following formula:

$$P = \frac{e^{-x} \cdot x^{r}}{r} \quad \text{where } r = \text{number of tumours in test group}, \; x = \text{mean number of tumours per group}$$

Table 4 *Lung tumours found and microscopically confirmed in first bichromate industry series*

Group No	*Test material*	*No rats in group*	*Bronchial carcinoma of left lung*	*Induction period in days (range)*	*Lung tumours not associated with treatment*
1	Ground chromate ore	100	0		
2	Bolton high lime residue	100	0		
3	Residue after alumina precipitation	100	0		
4	Residue from slurry tank—free of soluble Cr	100	0		
5	Residue from vanadium filter	100	0		Pulmonary adenoma of left lung
6	Residue from slurry disposal tank	101	0		Anaplastic carcinoma of upper left lung
7	Sodium dichromate dihydrate	100	0		Fibrosarcoma of upper left lung
8	Sodium chromate	100	0		
9	Chromic acid (ground)	100	1	560	
10	Chromic acid (metal)	100	0		
11	Calcium chromate	100	8†	604 (473–734)	
12	Chromic chloride hexahydrate	100	0		Lymphoma of right lung
13	Zinc chromate—type II*	100	3†	708 (657–734)	
14	Chrome tan	100	0		
15	Pellet + cholesterol	150	0		Adenoma of right lung
16	Blank pellet	150	0		Adenocarcinoma of right lung
17	100% 3-MCA	48	36	498 (270–734)	
18	50% 3-MCA	48	18	474 (284–696)	
19	25% 3-MCA	48	13	517 (297–698)	
20	100% 3-MCA	48	34	493 (217–730)	

*Zinc potassium chromate.
†Statistically significant at the 5% level.
MCA = 20-methylcholanthrene.

Zinc chromates

Although bronchial carcinomas were seen in all three groups of chromates containing zinc, only in group 6 (zinc chromate—low solubility) was the number (5/100) statistically significant. In group 14 (zinc chromate—Norge), which is of similar composition to group 6, the production of three bronchial carcinomas was not significant ($p = 0{\cdot}068$).

Previous studies performed using chromates of zinc need some care in interpretation since the identity of the zinc material used is uncertain.[26 27] Hueper's work in 1961 states that "zinc yellow" induced both injection site sarcoma and intrapleural tumours. Later reports, however, quote this same study as using zinc chromate hydroxide[28] or zinc yellow.[29] In this latter reference a footnote is added to the effect that "it is not certain whether the zinc yellow used in these studies was zinc chromate, zinc potassium chromate, or zinc yellow (a pigment based on zinc potassium chromate with a formula which approximates to $4ZnO{\cdot}K_2O{\cdot}4CrO_3{\cdot}3H_2O$)." A previous study by Levy using the zinc potassium chromate (zinc chromate—type II—$K_2CrO_4{\cdot}3ZnCrO_4{\cdot}Zn(OH)_2$ of 99–100% purity) gave 3/100 bronchial carcinomas (all squamous)[22]; and this result was statistically significant (table 4).

The single tumour recorded in group 7 (zinc tetroxychromate) is not easy to explain. Table 1 shows that there is a relatively low amount of chromium (8·8%) in this form of zinc chromate and this may in part account for its lack of activity.

Lead chromates

Within the seven lead chromate containing groups four were squamous carcinomas, one in each of groups 1, 2, 10, and 13 (table 3). These results are not statistically significant. Nevertheless, because of the rarity of this lesion in control rats it is important to consider their relevance. In previous studies with lead chromate pigments the precise form of lead chromate, which can vary considerably, has not always been stated, and intramuscular or subcutaneous injections have always been used.[26 30–32] Some of these studies have produced a high incidence of sarcomas at the site of injection[31 32]: the significance of this finding is uncertain.

It is thought that the present study more accurately represents the potential of lead chromate containing materials as possible lung carcinogens, and that lead chromate is non-carcinogenic or has an extremely low carcinogenic potential under the conditions used.

Barium chromate

No bronchial carcinomas were produced by this material in the present study. Hueper subjected rats to intramuscular or intrapleural implants of barium chromate; no tumours were observed in the intramuscular study and only one in the intrapleural study.[26] Although the animal data are limited, there is no evidence from this or other studies to suggest that barium chromate is carcinogenic.

Highly soluble chromates

Chromic acid produced two bronchial carcinomas (one squamous and one anaplastic carcinoma) and each was responsible for the death of the male rat in which it occurred. Previous studies by Laskin *et al* and Levy using the intrabronchial pellet technique produced 0/100 and 1/100 squamous carcinomas respectively with chromic acid.[22 26] The IARC considered the animal data insufficient for evaluation,[29] and from the result of the present study it may be concluded that chromic acid may, at most, evoke a weak carcinogenic response under the conditions of the bioassay.

Sodium dichromate tested by intramuscular or intrapleural injection,[33] or both, or intramuscular implantation[26] did not produce tumours in rats. The present result and that of an earlier intrabronchial pellet implantation study[22] suggests that sodium dichromate is not carcinogenic, or at most is an extremely weak carcinogen.

Bichromate: production process materials

Chemically, these materials are not closely related, but as they may be present at some stage in the process of bichromates production they are conveniently discussed together.

Studies using trivalent chrome ore have produced no evidence of carcinogenicity, even by the subcutaneous route, and it is thus concluded that the negative result for chrome ore (group 18 table 3) represents a true non-carcinogenic response in animal systems. In a previous study a material described as Bolton high lime residue did not produce any bronchial carcinomas.[22] Laskin *et al*, however, using the same technique induced a single squamous carcinoma in 100 treated rats, with a material described as "process residue."[25] It is of interest to note that group 16 material contained 2·7% $CaCrO_4$, the Bolton high lime residue contained 2·4% $CaCrO_4$, and the process residue used by Laskin *et al*[25] contained up to 3% $CaCrO_4$. It is concluded that a weak carcinogenic response, at most, may be represented by the single tumour seen in this and in the Laskin study and that it is most probably due to the $CaCrO_4$ content. The single bronchial carcinoma seen in group 17 (vanadium solids) and the two squamous carcinomas produced by group 19 (kiln frit—2% limestone in feedmix), represent a weak carcinogenic response due to the presence of calcium chromate. Group 20 (recycled residue—2% limestone in feedmix) gave no bronchial carcinomas and because the material contains only a small fraction of hexavalent chromium (see table 1) it is concluded that this is a true negative result.

CHROMATE CARCINOGENESIS AND INDUSTRIAL IMPLICATIONS

The main reason for the toxicological distinction between hexavalent and trivalent chromium is that hexavalent chromium (as the chromate ion) is readily transported into the cell[34-37] whereas trivalent chromium appears unable to cross cell membranes.[38 39] Once inside the cell, the chromate ion is enzymatically reduced to the biologically active trivalent form and it is Cr^{3+} which is able to form ligands with macromolecules, including DNA.[40] Reduction of Cr^{6+} to Cr^{3+} must occur close to the cellular DNA to allow formation of potentially damaging chromium complexes with critical DNA targets. If this reduction occurs in the extracellular fluids then the chromium will be unavailable for reaction with other molecules. This is consistent with the findings that trivalent chromium compounds have been found to be non-carcinogenic in animal studies, whereas certain hexavalent chromium materials are carcinogenic. It is believed, however, that many of the reported animal studies have not distinguished between the carcinogenic potential of hexavalent chromium containing materials with sufficient precision for useful advice to be given on the use and handling of chromates in the industrial context. As an example, the ready production of injection site sarcomas by single or repeated injections has not been particularly helpful in the interpretation of epidemiological data.

The model used in this and a previous study is aimed directly at the target tissue for man, the bronchial epithelium. The loaded implanted pellet as well as releasing the chromate ion presents a constant irritant stimulus (often thought a potentiator for neoplastic development). Factors that will determine the response of the rat lung will be the amount of chromate ion contained in a pellet, the rate of release of chromate ion to the target tissue, and the lipid/water interactions and lipoprotein penetration at the cell wall interface.

The lung tumour results show some interesting trends, particularly in relation to solubility, and if one considers principally the pure chromium compounds then it is apparent that neither the highly soluble (chromic acid or sodium dichromate) nor the insoluble (lead chromate pigments and barium chromate) can be considered to be overtly carcinogenic in the system. Only those chromates described as "sparingly" or of "medium" solubility (strontium, calcium, and zinc chromate) gave a frankly neoplastic response and the epidemiological evidence confirms this result. In the case of the bichromate production process materials the data are more difficult to interpret, but the limited conclusion that may be drawn is that if any of the process materials are carcinogenic under the conditions of this bioassay system it is probably those containing calcium chromate.

The results of this study of bichromate production materials, taken together with those of a previous study,[22] suggest that the introduction of the "no lime" process, with the consequent reduction in risk of calcium chromate exposure for bichromate workers, may partly explain the reduction in the risk of lung cancer seen in the industry.[41] Improved industrial hygiene must also be considered a contributory factor.

The findings for chromate pigments (lead and zinc chromates) and those of previous animal studies, may help to explain the reported lung cancer risk for chromate pigment workers. The workers in this industry tend to have a mixed exposure to both lead and zinc chromate, but the evidence of both animal and human studies strongly support the hypothesis that this risk may be entirely due to exposure to zinc chromates. This is confirmed by the epidemiological findings of Davies, who was able to show that workers did not experience an increased risk of lung cancer when exposed to lead chromate alone, even under conditions capable of evoking overt lead poisoning.[20 42]

The experimental model used here seems to yield results that are corroborated by epidemiological findings among groups of workers exposed to chromium containing materials and thus the results may be of value in estimating human risk.

We thank Ms Hilary Cross for preparing the histopathological material and also Dr R L Carter, consultant pathologist of the Royal Marsden Hospital, for his help in the pathological evaluation. We gratefully acknowledge the financial support of British Chrome and Chemicals in this and previous studies, the European Pigment Manufacturers Association, Dry Color Manufacturers Association (USA), and the Ciba-Geigy Corporation throughout this present study.

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